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A COMPARISON OF THE EFFECT OF MINERAL AND CHELATE FORMS OF COPPER, ZINC AND MANGANESE ON YIELD AND NUTRIENT STATUS OF GREENHOUSE LETTUCE

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Abstract. Consumption of vegetables with either too low or excessive contents of micronutrients may have an adverse effect on human health. In intensive horticultural production mineral fertilizers, containing mineral or chelate compounds with varying micronutrient fertilization efficiency, constitute the main sources of micronutrients for plants. In the years 2009–2010 experiments with lettuce were conducted to investigate the effect of mineral (sulfates) and chelate (IDHA chelate, EDTA + DTPA chelate) forms of copper, zinc and manganese on yield and micronutrient contents in leaves. Two levels of micronutrients were applied (mg· dm^{-3} of substrate): I. 5 Cu, 10 Zn, 10 Mn, and II. 20 Cu, 40 Zn and 40 Mn. Lettuce was grown in 6 dm^3 containers filled with limed highmoor peat, enriched with macro- and micronutrients. Lettuce was harvested at the consumption stage. Contents of Cu, Zn and Mn were determined by ASA in lettuce leaves. The volume of lettuce yield at a lower content of micronutrients in the substrate did not depend on the form in which copper, zinc and manganese were introduced. After the application of higher rates of micronutrients in the mineral or IDHA chelate forms a significantly higher crop was produced than in case of EDTA + DTPA chelates. An increase in micronutrient rates in the substrate had a significant effect on an increase in their contents in vegetables. At the nutrition of crops with micronutrients at higher rates in the form of EDTA + DTPA chelates a significant increase was found in copper content in lettuce. After the application of higher rates of micronutrients plants that were introduced two micronutrients in the IDHA chelate forms and one in the mineral form contained less zinc. Leaves of lettuce grown on substrate with mineral forms of copper, zinc and manganese contained significantly more manganese than those grown in substrate with chelate forms of micronutrients.

Key words: *Lactuca sativa* L., micronutriens, sulfates, chelate IDHA, chelate EDTA + DTPA

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INTRODUCTION

Both a deficit and excess of micronutrients in plants lead to disturbances of many physiological and biochemical processes, which results in a reduction of yields and first of all a deterioration of their nutritive value [Ruszkowska and Wojcieska-Wyskupajtys 1996, Grzyś 2004]. Concentrations of micronutrients in the soil solution and thus their availability to plants depend on soil, climatic and fertilization factors [Gębski 1998, Łabętowicz and Rutkowska 2001]. Micronutrients are supplied to plants mainly in mineral fertilizers, in which they may be found in mineral or chelate combinations of different efficiency. In fertilizers containing chelates microelements are bound through coordinate bonds with different coordination compounds, which protect cations against leaching or bounding in soil. Presently used chelators exhibit various biodegradability levels and some of them may be accumulated in the environment for many years, which is an adverse phenomenon [Hoffmann et al. 2004, Borowiec et al. 2007 and 2009, Villen et al. 2007, Huculak et al. 2009]. Many authors showed that in iron plant nutrition chelate forms are much more effective than mineral forms [Ylivainio et al. 2004 and 2006, Ojeda et al. 2004]. Few studies have been conducted to compare the efficiency of the action of mineral and chelate forms of other micronutrients in growing of horticultural plants.

The aim of this study was to assess the effect of copper, zinc and manganese applied in the form of sulfates, IDHA chelates or EDTA + DTPA chelates on fresh weight of plants as well as contents of micronutrients in leaves of greenhouse lettuce grown on highmoor peat.

MATERIAL AND METHODS

In the years 2009–2010 in the spring in an unheated greenhouse experiments were conducted on head lettuce cv. 'Michalina'. Experimental factors included:

A. forms of copper, zinc and manganese:
  1. mineral $CuSO_4 \cdot 5\ H_2O$, $ZnSO_4 \cdot 7\ H_2O$, $MnSO_4 \cdot H_2O$
  2. chelate a) CuIDHA, ZnIDHA, MnIDHA
     (IDHA chelate – imidodisuccinic acid)
     b) CuEDTA + DTPA, ZnEDTA + DTPA, MnEDTA + DTPA
     (EDTA + DTPA chelate – ethylenediaminetetraacetic acid + diethylene triamine pentaacetic acid)

B. the level of micronutrients ($mg \cdot dm^{-3}$ of substrate):
  1. 5 Cu, 10 Zn, 10 Mn
  2. 20 Cu, 40 Zn, 40 Mn

In individual combinations copper, zinc and manganese were introduced to the substrate in the mineral form, in the form of IDHA chelates, as EDTA + DTPA chelates or with two components in the chelate form and one in the mineral form. The design of the combinations is presented in tables 1–4.

Each combination was composed of 4 replications. A replication comprised a container with 4 lettuce plants. Single seeds of lettuce were sown on 18th March 2009 and

30th March 2010 to multiple trays with opening diameter of 4 × 4 cm. The applied substrate consisted of highmoor peat from Lithuania, limed at 5 g $CaCO_3 \cdot dm^{-3}$ and enriched with Azofoska (multi compound fertilizer) at 1.5 g· dm^{-3}. At the phase of two developed leaves (14.04.2009 and 21.04.2010) the seedlings were planted to containers of 6 dm^3 filled with adequately prepared substrate. The substrate comprised highmoor peat limed on the basis of a neutralization curve to pH 6.5 and enriched with nutrients in the form of saline solutions. After liming on the basis of a chemical composition of the substrate the contents of macro- and micronutrients were supplemented to the following levels (in mg · dm^{-3}): N – 180, P – 140, K – 220, B – 1, Mo – 1, Fe – 25, and Cu, Zn, Mn according to the experimental design. Initial content of microelements in substrate was (in mg · $dm^{-3}$of substrate): Cu – 0.4, Zn – 2.0, Mn – 2.7. Ca, Mg and S-SO_4 were not added to the substrate, since contents of these nutrients were Ca – 2045, Mg – 160 and S-SO_4 – 25 mg· dm^{-3}. In the vegetation period plants were watered to 70% water capacity of peat determined using Wahnschaff cylinders. Lettuce was harvested at the consumption stage (14.05.2009 and 25.05.2010). Plants were cut, then weighed and next dried in a convection dryer and homogenized. After wet mineralization of the plant material in a mixture of acids HNO_3 and $HClO_4$ at a ratio of 3:1 contents of Cu, Zn and Mn were determined according to ASA method.

Results concerning the yield of fresh weight of plants and micronutrient contents were subjected to statistical analysis using the analysis of variance for three-factorial experiments. After significant differences were found means were grouped according to the Newman-Keul's test at the significance level $\alpha = 0.05$.

RESULTS AND DISCUSSION

Fresh weight of lettuce heads after the application of copper, zinc and manganese at lower doses did not depend on the form of micronutrients (tab. 1). Yield of plants from those combinations was similar to that of lettuce obtained after plants were supplied micronutrients at higher doses in the mineral form or as IDHA chelates. The advantageous effect of micronutrients in the form of IDHA chelates was confirmed in a study by Lucena et al. [2008] on greenhouse growing of pea and tomato. In turn, in combinations with EDTA + DTPA chelates a significant reduction of yields of plants was observed. The biggest reduction of yield was caused by the introduction to the substrate of higher doses of copper, zinc and manganese or zinc and manganese in this form and copper in the form of sulfates. Similarly, Tyksiński and Komosa [2007], after the application of iron in the form of EDTA + DTPA chelate at doses ranging from 75 to 125 mg·dm^{-3} a significant reduction was found in terms of yield, which in the opinion of the authors was caused by the carriers used in the chelate. In the investigations conducted by the authors of this study no significant difference was found in the yields of lettuce between the years of analysis. In earlier studies on lettuce grown on peat Kozik et al. [2008 a, b; 2009] differentiated in individual experiments the forms and doses of individual micronutrients. In case of copper doses applied at 5 to 75 mg·dm^{-3} and manganese at 10 to 60 mg·dm^{-3} significantly higher mean yields of lettuce were obtained by the authors at the application of mineral forms of copper or manganese than the chelate forms (EDTA + DTPA) of those micronutrients. In turn, Chohura et al. [2004] showed a significant increase in yields of tomato grown in rockwool at the combined application of copper and manganese in the form of chelates (EDTA + DTPA) in comparison to the mineral forms. Kozik et al. [2009] did not find any differences in the yield of lettuce fertilized with chelate or mineral forms of zinc. Similarly in Boxma and Groot [1985] studies the form of applied Mn ($MnSO_4$, MnEDTA or MnDTPA) had no significant effect on yield of oats.

Table 1. Effect of different copper, zinc and manganese fertilization on yield of lettuce cv 'Michalina'
Tabela 1. Wpływ zróżnicowanego nawożenia miedzią, cynkiem i manganem na plon sałaty odmiany 'Michalina'

Combinations Kombinacje	Yield of lettuce – g · plant^{-1} f.m. Plon sałaty – g · roślina^{-1} św.m.					
	I dose – dawka		mean	II dose – dawka		mean
	year – rok			year – rok		
	2009	2010		2009	2010	
$CuSO_4$, $ZnSO_4$, $MnSO_4$	129.25	137.00	133.13d	125.50	138.00	132.25d
CuIDHA, ZnIDHA, MnIDHA	120.25	136.00	133.13d	121.25	142.25	131.75d
CuEDTA+DTPA, ZnEDTA+DTPA, MnEDTA+DTPA	126.75	131.75	129.25d	14.00	8.50	11.25a
$CuSO_4$, ZnIDHA, MnIDHA	131.50	138.75	135.13d	114.50	124.75	119.63d
CuIDHA, $ZnSO_4$, MnIDHA	136.25	142.00	139.13d	130.00	140.00	135.00d
CuIDHA, ZnIDHA, $MnSO_4$	121.00	131.25	126.13d	129.00	135.00	132.00d
$CuSO_4$, ZnEDTA+DTPA, MnEDTA+DTPA	117.25	121.75	119.50d	27.00	8.00	17.50a
CuEDTA+DTPA, $ZnSO_4$, MnEDTA+DTPA	133.00	114.50	123.75d	56.26	49.25	52.75b
CuEDTA+DTPA, ZnEDTA+DTPA, $MnSO_4$	130.25	114.75	122.50d	71.00	64.50	67.75c
Mean for dose – Średnia dla dawki			129.07b			88.88a
Mean for years – Średnia dla lat	2009 r. – 107.50a			2010 r. – 110.44a		

Dose – Dawka I: 5 mg Cu, 10 mg Zn, 10 mg Mn · dm^{-3}
Dose – Dawka II: 20 mg Cu, 40 mg Zn, 40 mg Mn · dm^{-3}
Means followed by same letters are not significantly different for $\alpha = 0.05$
Średnie oznaczone tymi samymi literami nie różnią się istotnie na poziomie $\alpha = 0,05$

Contents of copper, zinc and manganese in lettuce depended significantly on the year of the study as well as the doses and forms of micronutrients (tables 2–4). Plants grown in 2010 contained significantly more micronutrients than those grown in 2009. Irrespective of the year, both at lower and higher contents of microelements in the substrate, the highest content of copper was shown in plants fertilized with copper, zinc and manganese in the form of EDTA + DTPA chelates. Copper content was similar in leaves of lettuce grown on the substrate with mineral compounds of copper, zinc and manganese at both applied doses. Heitholt et al. [2002] after application of copper sulfate in the range from 0 to 100 ppm obtained similar copper content in the leaf blades of soybean grown in the greenhouse. Authors found that increased fertilization of copper sulfate significantly influenced the increase of Mn content in leaves. The lowest amounts of copper were recorded in plants after the application of copper in the form of sulfates, while those of zinc and manganese when applied as EDTA + DTPA chelates at lower doses. Kozik et al. [2008 b] stated that the content of copper in lettuce was two times higher after the application of copper as a chelate than in the mineral form. In the same study it was shown that with increasing doses of copper, irrespective of its form, its content in leaves increased. In turn, Chohura et al. [2004] did not obtain an increase in copper content in tomato leaves under the influence of copper application in the form of EDTA + DTPA chelate individually or jointly with manganese chelate. According to Kabata-Pendias and Pendias [1999], mean content of copper in aboveground parts of plants is 5–20 mg · kg $^{-1}$. In this study after the introduction to the substrate of higher doses of the three or two analyzed micronutrients as EDTA + DTPA chelates a higher copper content was recorded in plants.

Table 2. The copper content in lettuce depending on the form and dose of copper, zinc and manganese in the substrate
Tabela 2. Zawartość miedzi w sałacie w zależności od formy i dawki miedzi, cynku i manganu w podłożu

Combination Kombinacja	Cu content mg·kg^{-1} d.m. Zawartość Cu mg·kg^{-1} s.m.					
	I dose – dawka		mean	II dose – dawka		mean
	year – rok			year – rok		
	2009	2010		2009	2010	
$CuSO_4$, $ZnSO_4$, $MnSO_4$	10.89	6.80	8.89bc	11.07	7.28	9.17c
CuIDHA, ZnIDHA, MnIDHA	10.83	10.34	11.63d	20.96	20.23	20.59h
CuEDTA+DTPA, ZnEDTA+DTPA, MnEDTA+DTPA	11.88	12.38	12.13e	63.49	30.15	46.82l
$CuSO_4$, ZnIDHA, MnIDHA	9.39	7.58	8.48bc	13.52	14.53	14.02f
CuIDHA, $ZnSO_4$, MnIDHA	12.60	7.73	10.16d	14.67	18.43	16.55g
CuIDHA, ZnIDHA, $MnSO_4$	7.47	7.78	7.63ab	14.56	15.18	14.87f
$CuSO_4$, ZnEDTA+DTPA, MnEDTA+DTPA	7.56	6.93	7.24a	27.78	27.98	27.88j
CuEDTA+DTPA, $ZnSO_4$, MnEDTA+DTPA	7.71	9.40	8.56bc	35.88	22.63	29.25k
CuEDTA+DTPA, ZnEDTA+DTPA, $MnSO_4$	8.27	8.85	8.56bc	22.65	21.13	21.89i
Mean for dose – Średnia dla dawki			9.14a			22.34b
Mean for years – Średnia dla lat	2009 r. – 17.30b			2010 r. – 14.18a		

Explanation see Table 1.

After the application of three micronutrients in the mineral form the contents of zinc and manganese in plants were highest (tables 3 and 4). However, at higher contents of micronutrients in the substrate similar contents of zinc were found in plants when either the three micronutrient, or zinc and manganese were applied as EDTA + DTPA chelates and with copper used in the form of sulfates. At a lower content of micronutrients sig-

Table 3. The zinc content in lettuce depending on the form and dose of copper, zinc and manganese in the substrate
Tabela 3. Zawartość cynku w sałacie w zależności od formy i dawki miedzi, cynku i manganu w podłożu

Combination Kombinacja	Zn content mg·kg^{-1} d.m. – Zawartość Zn mg·kg^{-1} s.m.					
	I dose – dawka		mean	II dose – dawka		mean
	year – rok			year – rok		
	2009	2010		2009	2010	
$CuSO_4$, $ZnSO_4$, $MnSO_4$	131.07	98.10	114.59de	213.01	159.80	186.40g
CuIDHA, ZnIDHA, MnIDHA	98.09	68.75	83.42bc	128.35	134.93	131.64f
CuEDTA+DTPA, ZnEDTA+DTPA, MnEDTA+DTPA	88.20	70.20	79.20a-c	181.21	183.63	182.42g
$CuSO_4$, ZnIDHA, MnIDHA	93.22	80.58	86.90c	116.03	108.18	112.10de
CuIDHA, $ZnSO_4$, MnIDHA	87.04	69.80	78.42a-c	112.87	105.93	109.40d
CuIDHA, ZnIDHA, MnSO4	90.77	79.08	84.92bc	121.85	112.03	116.94de
$CuSO_4$, ZnEDTA+DTPA, MnEDTA+DTPA	73.41	73.20	73.31a	143.19	216.40	179.79g
CuEDTA+DTPA, $ZnSO_4$, MnEDTA+DTPA	73.27	67.78	70.52a	136.47	138.48	137.47f
CuEDTA+DTPA, ZnEDTA+DTPA, $MnSO_4$	73.03	79.84	76.43ab	123.23	117.08	120.15e
Mean for dose – Średnia dla dawki			83.08a			141.81b
Mean for years – Średnia dla lat	2009 r. – 115.79b			2010 r. – 109.10a		

Explanation see Table 1.

Table 4. The manganese content in lettuce depending on the form and dose of copper, zinc and manganese in the substrate
Tabela 4. Zawartość manganu w sałacie w zależności od formy i dawki miedzi, cynku i manganu w podłożu

Combination Kombinacja	Mn content mg·kg^{-1} d.m. – Zawartość Mn mg·kg^{-1} s.m.					
	I dose – dawka		mean	II dose – dawka		mean
	year – rok			year – rok		
	2009	2010		2009	2010	
$CuSO_4$, $ZnSO_4$, $MnSO_4$	166.04	161.50	163.77d	308.51	360.70	334.61h
CuIDHA, ZnIDHA, MnIDHA	112.34	104.10	108.22b	192.42	213.80	203.11f
CuEDTA+DTPA, ZnEDTA+DTPA, MnEDTA+DTPA	85.42	86.33	85.87a	162.24	86.70	124.47bc
$CuSO_4$, ZnIDHA, MnIDHA	115.42	117.73	116.57bc	175.93	187.05	181.49e
CuIDHA, $ZnSO_4$, MnIDHA	108.35	121.43	114.89bc	205.84	221.77	213.81fg
CuIDHA, ZnIDHA, $MnSO_4$	126.89	120.70	123.79bc	223.32	212.90	218.11g
$CuSO_4$, ZnEDTA+DTPA, MnEDTA+DTPA	92.02	96.08	94.05a	138.99	96.28	117.63bc
CuEDTA+DTPA, $ZnSO_4$, MnEDTA+DTPA	104.70	80.95	92.83a	151.52	100.70	126.11c
CuEDTA+DTPA, ZnEDTA+DTPA, $MnSO_4$	86.06	85.68	85.87a	141.12	117.00	129.09c
Mean for dose – Średnia dla dawki			109.54a			183.15b
Mean for years – Średnia dla lat	2009 r. – 149.84b			2010 r. – 142.85a		

Explanation see Table 1.

nificantly lower amounts of zinc and manganese were recorded in leaves of lettuce in the combinations, in which three or two components were used as EDTA + DTPA chelates. Similar contents of zinc were found in lettuce fed zinc in the form of sulfates, while copper and manganese were supplied as IDHA chelates. At higher doses of micronutrients lower levels of zinc were contained in plants, when two micronutrients were introduced to the substrate in the form of IDHA chelates and one as sulfates, while less manganese was found when three or two nutrients were applied as EDTA + DTPA chelates. Kozik et al. [2008 a and 2009] reported higher contents of zinc and manganese in leaves of lettuce fed those micronutrients in the mineral form than as EDTA + DTPA chelates. In turn, Chohura et al. [2004] stated that after the application of manganese in the form of EDTA + DTPA chelate the content of this nutrient in tomato leaves was higher than after the application of manganese sulfate. Similarly, Spiak [1996] recorded lower contents of zinc in oats and mustard and Prasad and Sinha [1981] in corn, after the application of zinc in the mineral form than as EDTA chelate. Consumption of lettuce which contains more than 20 mg Cu $\cdot$ kg^{-1} d.m. can be harmful for health. This survey indicate that the dose of EDTA + DTPA chelate as a source of micronutrients should be smaller. Micronutrients content in lettuce obtained in the other combinations do not raise objections from the standpoint of human nutrition. That indicate the standard recommendations of daily intake of Cu, Zn [Ziemlański 2001] and Mn [Kabata-Pendias and Pendias 1999] and the percentage share of lettuce in daily diet.

CONCLUSIONS

1. The volume of lettuce yield at lower contents of micronutrients in the substrate (5 mg Cu, 10 mg Zn, 10 mg Mn $\cdot dm^{-3}$) did not depend on the form in which copper, zinc and manganese were introduced.
2. An increase in micronutrient doses in the substrate (20 mg Cu, 40 mg Zn, 40 mg Mn $\cdot$ dm^{-3}) in the form of EDTA + DTPA chelates resulted in a reduction of yield and an increase in the content of copper in plants.
3. At a higher micronutrients dose plants contained less zinc when two micronutrients were introduced to the substrate as IDHA chelates and one in the form of sulfates.
4. Leaves of lettuce grown in the substrate with mineral forms of copper, zinc and manganese contained significantly more manganese than those grown in the substrate with chelate forms of micronutrients.

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PORÓWNANIE WPŁYWU MINERALNYCH I CHELATOWYCH FORM MIEDZI, CYNKU I MANGANU NA PLON ORAZ STAN ODŻYWIENIA SAŁATY SZKLARNIOWEJ

Streszczenie. Spożywanie warzyw o zbyt małej lub o nadmiernej zawartości mikroskładników może niekorzystnie wpłynąć na zdrowie człowieka. W intensywnej produkcji ogrodniczej głównym źródłem mikroskładników dla roślin są nawozy mineralne, zawierające mineralne lub chelatowe związki o zróżnicowanej skuteczności dostarczania mikroskładników roślinom. W latach 2009–2010 w doświadczeniach z sałatą oceniono wpływ mineralnych (siarczany) i chelatowych (chelat IDHA, chelat EDTA + DTPA) form miedzi, cynku i manganu na plon oraz zawartość mikroskładników w liściach. Zastosowano dwa poziomy mikroskładników (mg· dm^{-3} podłoża) : I. 5 Cu, 10 Zn, 10 Mn; II. 20 Cu, 40 Zn, 40 Mn. Sałatę uprawiano w pojemnikach o objętości 6 dm^3, wypełnionych torfem wysokim zwapnowanym i wzbogaconym w makro- i mikroskładniki. Zbiór sałaty przeprowadzono w fazie dojrzałości konsumpcyjnej. W liściach sałaty oznaczono zawartość Cu, Zn i Mn metodą ASA. Wielkość plonu sałaty przy mniejszej zawartości mikroskładników w podłożu nie zależała od formy, w jakiej zostały wprowadzone miedź, cynk i mangan. Po zastosowaniu większej dawki mikroskładników w formie mineralnej lub chelatów IDHA uzyskano istotnie większy plon roślin niż w formie chelatów EDTA + DTPA. Zwiększenie dawki mikroskładników w podłożu istotnie wpłynęło na wzrost ich zawartości w roślinach. Przy żywieniu roślin mikroskładnikami, w większych dawkach, w formie chelatów EDTA + DTPA stwierdzono istotne zwiększenie zawartości miedzi w sałacie. Po zastosowaniu większej dawki mikroskładników, mniej cynku zawierały rośliny, gdy wprowadzono do podłoża dwa mikroskładniki w formie chelatów IDHA, a jeden w formie mineralnej. Liście sałaty uprawianej w podłożu z mineralnymi formami miedzi, cynku i manganu zawierały istotnie więcej manganu niż w podłożu z chelatowymi formami mikroskładników.

Słowa kluczowe: *Lactuca sativa* L., mikroskładniki, siarczany, chelat IDHA, chelat EDTA + DTPA

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